11th Congress Int. Society Blood Transfusion, Sydney 1966
Edited by L. P. HOLLÄNDER, Basel
S. KARGER – BASEL/NEW YORK (Printed in Switzerland)
SEPARATUM

Proc. 11th Congr. int. Soc. Blood Transf., Sydney 1966; Bibl. haemat., No. 29, Part 3, pp. 735–738 (Karger, Basel/New York 1968)

From the U. S. Naval Hospital, Chelsea, Massachusetts

Use of Frozen Blood in Vietnam

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The United States Navy's Bureau of Medicine and Surgery has established frozen blood banks at the Naval Station Hospital, Danang, South-Vietnam and aboard the hospital ship, USS Repose. The purpose of this project is to test the practicability of using frozen blood under combat conditions as a supplement to ACD whole blood.

For the past two years, the Naval Blood Research Laboratory, Chelsea, Massachusetts, has conducted extensive clinical evaluations on human red cells which were preserved with glycerol and the slow-freeze and thaw technic, agglomeration being used to recover the preserved cells [2].

Small aliquot (10cc) autologous chromium labeled red cells were transfused to healthy volunteers in an attempt to define the pre-freeze, frozen-state and post-thaw variables, agglomeration being used to recover the deglycerolized red cells [5, 6]. Multiple full unit homologous transfusions were studied in stable, medical recipients [1]. *In vivo* survivals were measured by both the manual Ashby technic and a modified differential agglutination technic using the Technicon Auto-Analyzer [4].

Because of the relative simplicity of the agglomeration process and the encouraging results obtained from both the autotransfusions and the homologous transfusions, the agglomeration method of recovering the deglycerolized red cells was selected for evaluation in this field test.

The technical objectives of this feasibility study were the field testing of both the equipment and technic of HUGGINS in reconstituting frozen glycerolized red cells, and the evaluation of logistic problems concerned with supporting the operation of the frozen blood bank.

Approximately 500 units of selected red cells (0 cde/cde, K^-, Fya^-; 0 CDe/CDe, K^-, Fya^-) were collected with the cooperation of the Massachusetts Chapter of the American Red Cross, the Massachusetts General

Hospital, Boston, Massachusetts, and the Naval Hospitals, Beaufort, South Carolina and Chelsea, Massachusetts.

These units were glycerolized within five days of collection in ACD (NIH A) using 0.3% Na_2 EDTA. The addition of EDTA prevented the development of Coombs positive red cells previously observed [5]. The glycerolized red cells were stored at —80 °C and shipped in polystyrene foam containers with dry ice to Danang, South Vietnam and to the hospital ship, USS Repose, via Oakland, California and Subic Bay, Philippines; all of these sites have —80 °C refrigeration units.

Higher storage temperatures (+4 °C, —20 °C, and —30 °C) were evaluated in anticipation of the consequences of a power failure and in order to ascertain the suitability of more commonly available modes of refrigeration [7]. Excessive *in vitro* loss of cellular hemoglobin and unacceptable posttransfusion chromium survival were observed when autologous erythrocytes were stored at +4 °C for longer than 24 h, at —20 °C for longer than 3 days, and at —30 °C for longer than 7 days between periods of storage at —80 °C. These studies stressed the need for maintaining the red cells at —80 °C and indicated that this temperature could be maintained with dry ice. With the agglomeration method dry ice, or another refrigerant for maintaining —80 °C temperature, must be available at all times in case of a power failure.

The evaluation of alternative short-term storage temperatures revealed that the agglomeration per se was an excellent quality control aspect of the process. With good agglomeration, one can wash the glycerolized red cell mass on three separate occasions within 25 min. Failure to agglomerate is usually an indication that the ionic environment is too high. With poor preservation, the red cells leak intracellular electrolytes; this produces an increase in the ionic strength of the environment and prevents agglomeration [3].

If poor agglomeration is observed and if all other possible causes for this can be excluded, then one can assume that the storage temperature has risen to a critical level. If the red cells agglomerate poorly and the time for the three separate washings has been excessive, then the red cells should not be transfused.

Another attractive feature of this process is the centrifugation step prior to transfusion which removes supernatant fluid containing the products of hemolysis, especially free supernatant hemoglobin. These frozen red cells have been carefully evaluated through repetition of the systematic observations made during the studies of the stable, anemic patients. ACD collected and stored blood has been studied in a similar manner.

Forty-three patients received a total of 347 units of ACD and 307 units of frozen blood at Danang, South-Vietnam. The *in vitro* loss related to processing the frozen units was 26.7%, with a S.D. of $\pm 12.8\%$. The units of blood transfused to each patient were grouped within 24-h transfusion periods. The units transfused within each 24-h period were evaluated collectively. Each patient had one or more 24-h transfusion period and, during each, received either frozen or ACD blood, or both.

No significant differences ($p > 0.05$) were observed in the patients between frozen red cells and non-frozen cells with respect to the elevations of plasma hemoglobin, bilirubin (total and indirect), platelet counts, urine hemoglobin or serum creatinine. The mechanism of removal of the compatible non-viable cells for both the frozen and non-frozen red cells was not associated with hemoglobinemia. Hemolytic transfusion reactions were not observed with the transfusion of frozen red cells. In 8 patients transfused with ACD blood, hemolytic and non-hemolytic transfusion reactions were observed. The mechanism of the hemolytic transfusion reactions was related to [1] isoagglutinin titers of anti-A and anti-B transfused with group 0, ACD blood to A- and B-recipients, and [2] Rh-positive red cells to previously sensitized Rh negative recipients.

Frozen red cells have been used successfully to supplement the ACD liquid-preservative program presently being used in South-Vietnam. It should be stressed that the role of the frozen blood is only to supplement the liquid preservative program. At this time, the indications for use of the frozen cells appear to be in the following situations:

1. Massive transfusions (greater than 20 units). Frozen red cells can be generated to help control the massive hemorrhage.

2. Rh-negative recipients. Selection of Rh-negative red cells for freezing provide a ready source of this cell type. Sensitization of Rh-negative recipient with Rh-positive red cells has been observed frequently.

3. Supplement the ACD program. When the supply of blood is low, frozen red cells can be used.

4. Previously frozen, washed 0-negative and 0-positive blood contains significantly reduced titers of anti-A and anti-B isoagglutinins. Post-thaw washing significantly reduces these titers, and type 0 Rh-positive and Rh-negative deglycerolized red cells can be transfused to A- and B-recipients safely.

Finally, the logistic problems of supplying the frozen red cells have been minimal. The major logistic problems have been with supplying the 6750 cm^3 of wash solutions required for processing one unit of frozen red cells.

The opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Navy Department or the Naval Service at large.

References

1. ALMOND, D. V. and VALERI, C. R.: Observations on deglycerolized, agglomerated erythrocytes transfused in multiple units to stable, anemic patients. Transfusion *7:* 95 (1967).
2. HUGGINS, C. E.: Frozen Blood. J. amer. med. Ass. *193:* 941 (1965).
3. RUNCK, A. H.; VALERI, C. R. and SAMPSON, W. T.: Comparison of the effects of ionic and non ionic solutions on the volume and intracellular potassium of frozen and non frozen human red cells. Transfusion (Jan.–Febr. 1968).
4. SZYMANSKI, I. O.; VALERI, C. R.; ALMOND, D. V.; EMERSON, C. E. and ROSENFIELD, R. E.: Automated differential agglutination for measurement of red cell survival. Brit. J. Haemat. Suppl. 40. 1961.
5. VALERI, C. R.: The *in vivo* survival of Coombs positive autologous erythrocytes produced by agglomeration. Transfusion *6:* 247 (1966).
6. VALERI, C. R. and BOND, J. C.: Observations on the preservation of autologous human erythrocytes using glycerol, slow-freeze technic, and agglomeration. Transfusion *6:* 254 (1966).
7. VALERI, C. R.; RUNCK, A. H. and MCCALLUM, L. E.: Observations on autologous, previously frozen, deglycerolyzed, agglomerated, resuspended red cells. I. Effect of storage temperatures. II. Effect of adenine supplementation of glycerolized red cells prior to freezing. Transfusion *7:* 105 (1967).

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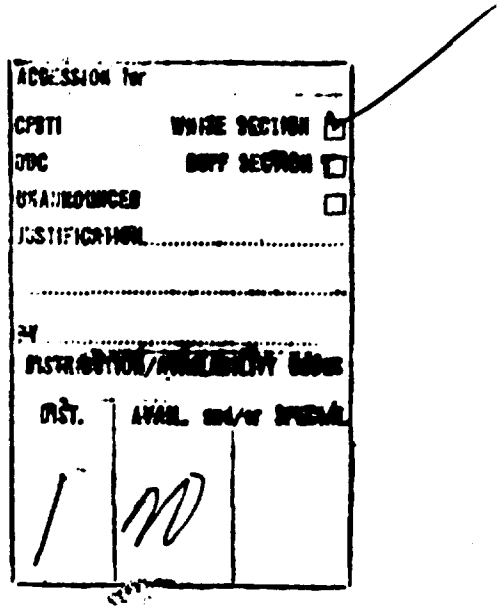

UNCLASSIFIED

Security Classification

AD687969

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Naval Blood Research Laboratory
Chelsea, Massachusetts 02150

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
USE OF FROZEN BLOOD IN VIETNAM

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(First name, middle initial, last name)*
CDR C. R. Valeri, MC, USNR, CDR C. E. Brodine, MC, USN, and LCDR G. S. Moss, MC, USNR

6. REPORT DATE
1966

7a. TOTAL NO. OF PAGES
4

7b. NO. OF REFS
7

8a. CONTRACT OR GRANT NO.
Program element: 63706N

b. PROJECT NO. M4305

c. Task area number: .02
Work unit number: 2001

d.

9a. ORIGINATOR'S REPORT NUMBER(S)

9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*

10. DISTRIBUTION STATEMENT
No. 1

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Bureau of Medicine and Surgery
Department of the Navy
Washington, D. C. 20390

13. ABSTRACT

Frozen red cells have been used successfully to supplement the ACD liquid preservative program presently being used in South Vietnam. It should be stressed that the role of the frozen blood is only to supplement the liquid preservative program. At this time, the indications for use of the frozen cells appear to be in the following situations: (U)

1. Massive transfusions (greater than 20 units). Frozen red cells can be generated to help control the massive hemorrhage.

2. Rh-negative recipients. Selection of Rh-negative red cells for freezing provide a ready source of this cell type. Sensitization of Rh-negative recipient with Rh-positive red cells has been observed frequently.

3. Supplement the ACD program. When the supply of blood is low, frozen red cells can be used.

4. Previously frozen, washed O-negative and O-positive blood contains significantly reduced titers of anti-A and anti-B isoagglutinins. Post-thaw washing significantly reduces these titers, and type O Rh-positive and Rh-negative deglycerolized red cells can be transfused to A- and B-recipients safely.

Finally, the logistic problems of supplying the frozen red cells have been minimal. The major logistic problems have been with supplying the 6750 cm^3 of wash solutions required for processing one unit of frozen red cells.

DD FORM 1 NOV 65 1473 (PAGE 1)

S/N 0101-807-6801

Unclassified

Security Classification